Confirmation of Opiates & Opioids According to SAMHSA and DOT

Hydrolysis with Finden BGTurbo® Enzyme

Finden, from Kura Biotech

Overview

Quantitation of opiates and opioids is particularly challenging. On one hand within the analytes that belong to this drug-class, we find some of the hardest to cleave glucuronides, on the other hand some of the free (unconjugated) analytes are particularly unstable and tend to bind to proteins or absorb into surfaces. In order to face this complex scenario, it is necessary that the hydrolysis provides fast, clean and highly specific conditions. This is all the more challenging than SAMHSA and DOT-compliant assays require to achieve an Upper limit of quantitation (ULOQ) of at least 15,000 ng/ml.

Genetically enhanced Kura BGTurbo® enzyme provides an efficient hydrolytic activity for the broad spectrum of conjugated analytes. Based on its specific affinity with “hard-to-cleave” glucuronides and its purity, BGTurbo® delivers optimum conditions for a complete and fast recovery of analytes being compatible with D&S methods due to its purity, without needing additional clean-up steps, or hydrolysis can be performed directly on Tip on Tip technology as well as filter plates being integrated into the sample prep, or using a Support Liquid Extraction.

Compatible Methods

Dilute & Shoot: This method is simple to implement and cost-effective. However, an additional clean-up step, like a protein crash followed by centrifuging, is often applied to eliminate the protein load added by traditional β-glucuronidase preparations. In practice, achieving desired sensitivity and reproducibility can be challenging. BGTurbo® presents very high glucuronidase activity at very low concentrations of enzyme, becoming compatible with D&S method, avoiding column clogging and interfering peak formation.

Filter & Shoot: Filter cartridges contain a solid phase sorbent bed and small pore frits. Positive pressure is used to prepare urine samples for LC-MS/MS analysis. This methodology eliminates centrifugation steps and removes particulates greater than 1 µm. Samples can be diluted at a ratio as low as 1:1, which is useful for detecting analytes at very low concentrations, providing higher sensitivity than dilute and shoot method. With BGTurbo® hydrolysis can be performed directly on the filter plates.

Solid Phase Extraction and Supported Liquid Extraction: These techniques are designed for rapid and selective sample preparation and purification prior to chromatographic analysis. During the last years, these techniques have been simplified on Automated Liquid Dispensers (ALD´s). However, hydrolysis, which is upstream of extraction, has been treated separately as an off-line manual process. BGTurbo® solves this bottleneck, not only does it provide flash hydrolysis but enables it to integrate and run hydrolysis directly on SPE/SLE plates in line with the extraction and LC-MS process, becoming a fully integrated sample preparation.

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1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448

Objectives

Table 1.

ANALYTICAL OBJECTIVE	INVOLVED ANALYTES	SOLUTION/TECHNICAL REQUIREMENT
Hydrolyze hard to cleave glucuronides	Dihydrocodeine-6-Glucuronide Codeine-6-Glucuronide Morphine-6-Glucuronide Tapentadol-Glucuronide Oxymorphone-3-Glucuronide Norbuprenorphine-3-Glucuronide	✔ Hydrolysis-recovery > 80% (ULOQ: 15,000 ng/mL). ✔ High affinity β-glucuronidase.
Protect labile analytes	6-Monoacetylmorphine	✔ Mild incubation temperature ✔ Sample prep. time below 60 minutes.
Avoid protein binding	Fentanyl, Morphine, Oxycodone	✔ Purified β-glucuronidase ✔ Low protein-content ✔ Short incubation time
Detect low concentration analytes	Fentanyl, (Nor) Buprenorphine, 6-MAM	✔ Low added background-noise

- Simplify and potentially automate workflow
- This highly purified enzyme doesn't need post hydrolysis clean-up.
- Preserve the integrity of labile analytes. [6-MAM, Benzodiazepines, (Synthetic) Cannabinoids].
- Extend column lifetime due to low protein enzyme and filter system.

BGTurbo® Hydrolysis Protocol

1. Optional: Centrifuge urine sample for 5 minutes at 4°C at 20,000 x g.
2. With a pipette, add 50 µL of urine sample to plate or column.
3. Add Instant Buffer I + BGTurbo® + ISDs + distilled water to the urine sample according to Table 1.
4. Mix by slowly inverting a capped test tube. If an automated pipetting station is used mixing can be done by repeating aspirate/dispense actions.
5. Incubate at 50°C for 10/30 minutes.
6. Proceed with the preferred extraction method.

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Table 2. Hydrolysis Mix Composition

Compound	10 minutes	30 minutes
	Volume (µL)	Volume (µL)
Urine	50	50
Instant Buffer I	20	20
BGTurbo Enzyme	**50**	**20**
Distilled Water	15	45
Internal Standards (50% - 100% MeOH)	15	15
Total	150	150
Incubation 50°		

Notes

- The protocol above is based on an initial volume of 50 µL of urine. The mix could be adapted to any required urine volume by keeping the given proportions.
- It's important to keep a minimum enzyme: urine ratio of 1:1 / 2:5 in order to achieve expected recoveries within 10/30 minutes, in spiked urine and mainly in authentic specimens.
- BGTurbo® is active from 0-20% MeOH but is optimal from 5 to 15% in the total hydrolysis mix.
- Mastermix containing Instant Buffer I, enzyme, DI-water and ISDs can be prepared to simplify workflow. Store at 2–8°C. Use within 14 days.

Testing & Validation

Table 3. Hydrolysis Control

Drug-Class	Recommended Hydrolysis Control (15,000 ng/mL of parent drug)
Opiates	Codeine-6-Glucuronide Morphine-3-Glucuronide 6-Monoacetylmorphine*
Opioids	Oxymorphone-3-Glucuronide Norbuprenorphine-3-Glucuronide Tapentadol Fentanyl**

*To check Integrity of this non glucuronidated analyte.
** To demonstrate absence of protein binding

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- Kura Biotech recommends performing validation in two steps:
  1. Run assay with spiked urine, using the hydrolysis controls mentioned above.
  2. Benchmark with authentic specimens.
  3. As a hydrolysis control, use codeine-6-glucuronide. (Hardest to cleave analyte).

Learn More

- BGTurbo® Datasheet
- Quick Start Guide BGTurbo®

References

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U.S. Patent Nos. 20180067116 and 202117324067 are still pending. United Kingdom Patent Nos.GB2553142 patent are granted.

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+1 800 332 1448